PB81-222697

Biosorption of Hydrophobic Organic Pollutants by Mixed Microbial Populations

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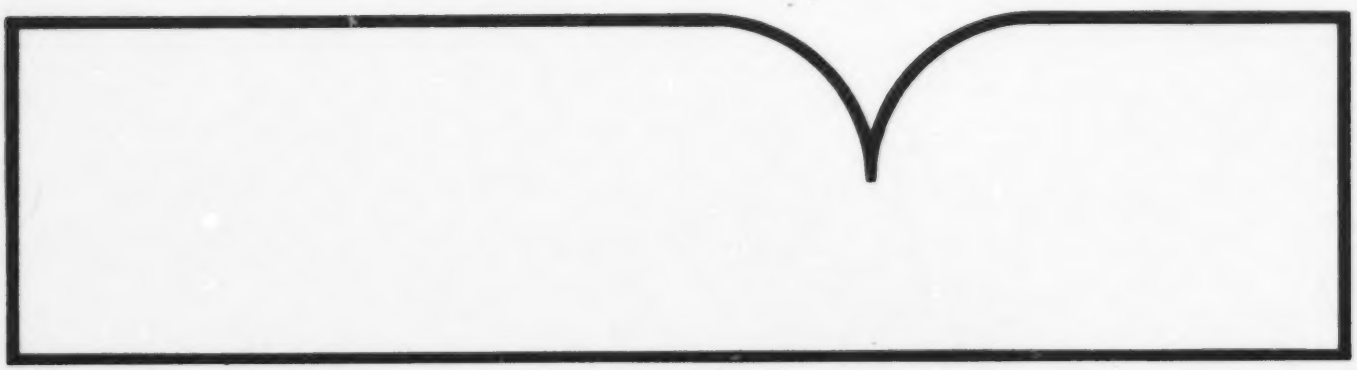

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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before complet

1. REPORT NO.	2.	3.
EPA-600/J-81-200	Journal Article	PB81-222697

4. TITLE AND SUBTITLE	5. REPORT DATE
Biosorption of Hydrophobic Organic Pollutants by Mixed Microbial Populations	May 1981
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
William C. Steen and Samuel W. Karickhoff	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Environmental Research Laboratory U.S. Environmental Protection Agency College Station Road Athens GA 30613	ACUL1A, ACBE1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U.S. Environmental Protection Agency--Athens GA Office of Research and Development Environmental Research Laboratory Athens GA 30613	Journal Article
	14. SPONSORING AGENCY CODE EPA/600/01

15. SUPPLEMENTARY NOTES
Published in Chemosphere 10(1):27-32, 1981
Contact: W.C. Steen, 250-3188

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17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	6
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (9-73)

Chemosphere Vol. 10, pp 27 - 32 0045-6535/81/010027-06$02.00/0

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ABSTRACT -- In recognition of the need to estimate biosorption for natural microbial populations, the variability of partition coefficients for two hydrophobic pollutants to natural populations from a variety of aquatic systems was investigated. Biosorption partition coefficients for pyrene [$2.46(\pm 0.6) \times 10^4$] and phenanthrene [$6.34(\pm 1) \times 10^3$] were nearly constant over 14 different microbial sources, consisting of sediments and soils from eight states. For these condensed ring aromatics, semi-empirical equations were developed relating biosorption partition coefficients to octanol/water partition coefficients and to water solubility and melting point. Concepts and relationships developed for these materials should extend to other families of hydrophobic compounds.

INTRODUCTION

Microbial biosorption or bioaccumulation of aquatic pollutants has received increased attention because of its impact on the environmental fate and effects of hazardous organics in aquatic systems [see recent review by Baughman and Paris (1)].

In recognition of the need to estimate biosorption for natural microbial populations, this study examines the variability of biosorption partition coefficients for specified hydrophobic organic compounds to natural populations generated from an aqueous suspension of soil and sediments. Partition coefficients for pyrene and phenanthrene were determined on mixed populations derived from natural sediments and soils collected in North Dakota, South Dakota, Iowa, West Virginia, Illinois, Indiana, Kentucky, and Georgia.

MATERIALS AND METHODS

Mixed microbial populations were generated from sediments and soils collected by Hassett and Banwart (2) and BY EPA personnel. Table I contains a brief description of the sediment/-soil source from which test populations were generated; for a more complete description, see Hassett et al. (2). Microbial populations were prepared by suspending 10 g (air-dry) of each sediment/soil in 500 ml of sterile 1% nutrient broth followed by incubation on a gyratory shaker for 48 hours. (28 ± 1°C). At the end of this growth period, the aqueous phase containing the microbial population was removed by siphoning, collected by centrifugation, and resuspended in sterile deionized water for sorption studies. Direct microscopic examination revealed a predominance of bacteria in the mixed microbial population. Pyrene and phenanthrene (analytical grade, K&K) were used as received from the manufacturer.

Sorption isotherms were determined by equilibrating aliquots of the stock bacterial suspensions with variable concentrations of aqueous solutions of pyrene or phenanthrene at 25°C. Each isotherm consisted of sorption measurements at three different concentrations of the test compounds, spanning in range approximately one order of magnitude.

Table I. Sources of Mixed Microbial Populations

No.[a]	Location	%o.c.
4	Missouri R., Stanton, ND	2.07
5	Missouri R., Lake Oahe, ND	2.28
6	Missouri R., Big Bend Lake, SD	0.72
8	Missouri R., Onawa, IA	0.15
9	Loess sample, Turin, IA	0.11
14	Soil eroded hillside, Ceredo, WV	0.48
15	Ohio R., Leavenworth, IN	0.95
18	Mississippi R., Columbus, KY	0.66
20	Soil, Fern Clyffe State Park, IL	1.30
21	Illinois R., Lorenzo, IL	1.88
22	Illinois R., Lacon, IL	1.67
23	Confluence of Illinois R. and Sangamon R.	2.38
26	Mississippi R., McClure IL	1.48
H.H.	Hickory Hill Pond, Athens, GA	1.40

[a] Samples 4-26 collected and characterized by John Hassett and Wayne Banwart (2). H.H. - Collected and characterized by local EPA personnel.

Bacterial concentrations were held constant for a given isotherm but the range varied between 10^{-4} and 10^{-5} g/ml (dry-weight basis) for different isotherms. Each isotherm was replicated four times.

For both compounds, sorption equilibration, as judged by sequential sampling, was complete within 30 minutes. All suspensions were allowed to equilibrate for 2 hours, however, with no measurable degradation of either compound. Subsequent to equilibration, the suspensions were centrifuged at 10K (Sorvall SS-34 rotor) for 15 minutes. Pyrene and phenanthrene concentrations remaining in the aqueous phase were determined by hexane extraction and uv absorption spectroscopic analysis. Sorbed concentrations were computed by measuring loss of compound from the aqueous phase. Mass balance was checked on one of each replicated set by direct extraction of the microbial pellet with hexane followed by solute analysis by uv absorption. Total recoveries ranged from 85 to 99% of the amounts added.

RESULTS AND DISCUSSION

Adsorption isotherms for all mixed bacterial populations were linear over the concentration range studied. That is, the sorbed concentration, C_b, at equilibrium (referenced to dry bacterial mass) was directly proportional to the solution phase solute concentration, C_w, with the proportionality constant being the partition coefficient, K_b.

$$C_b = K_b C_w \qquad (1)$$

Typical ranges in aqueous concentration, C_w, for a given isotherm were 1 to 20 ppb for pyrene and 30 to 300 ppb for phenanthrene. For a given isotherm, deviations in K_bs, replicated at a given concentration, approximated deviations in K_b measured at different concentrations. Table II contains the partition coefficients and regression coefficients for the isotherms least-squares fitted to eq. 1. [Linear least squares fitting through the origin was according to Gillingham and Heien (3).]

For a given compound, K_b was nearly constant for the different bacterial sources and independent of being derived from air dry or wet sediments (Hickory Hill). Pyrene K_bs ranged from 14,800 to 32,700 with a mean of 24,600 and coefficient of variation of 0.245. (Coefficient of variation = standard deviation/mean.) The isotherms with higher K_bs (samples 14,20,26) were characterized by greater data scatter (lower coefficients of determination) and/or poor mass balance. Isotherm replicates, where both sorbed and solution concentrations were measured, gave K_bs less than 27,000. Phenanthrene K_bs ranged from 5,220 to 7,530 with a mean of 6,340 and a coefficient of variation of 0.158.

Table II. Partition Coefficients for Pyrene and Phenanthrene

Bacteria Source	Pyrene $K_b(x10^{-4})$	R^2	Phenanthrene $K_b(x\ 10^{-3})$	R^2
5	1.48	0.98	5.22	0.98
6	2.10	0.98		
8	2.41	0.97	5.90	0.98
9	1.71	0.98		
14	3.26	0.95	7.53	0.98
20	3.27	0.95		
21	2.28	0.97		
22	2.36	0.98		
23	2.45	0.97		
26	3.07	0.95		
HH	2.00	0.96	6.70	0.97
Mean	$K_b = 2.46 \pm 0.6$		$K_b = 6.34 \pm 1$	

Although no concerted effort was made to generate microbial populations that accurately represent natural populations from given sources, the wide variety of sediment/soil sources (geographical and hydrological) should provide microbial populations indicative of diversity in natural occurrence. If we set as a goal K_b estimation within a factor of two, then variation due to source specificity can be ignored for pyrene and phenanthrene, and, therefore, be logically generalized to other similar compounds.

Estimation of K_b from physical properties

Independence of K_b on the source of the bacterial population greatly simplifies K_b estimation for aquatic systems, requiring a single K_b for each compound with no system specificity.

Bohonos et al. (4) have measured K_bs on mixed bacterial populations for other condensed ring aromatic compounds. Table III summarizes their sorption data along with other physical properties for these compounds. Bohonos and others [see Baughman and Paris (1) for review] have shown that sorption of these types of compounds is largely unaltered by killing the organisms, thus indicating "passive" binding in the sorbed state. The sorption process can be viewed as a physical partitioning of the organic compounds between water and an organic matrix. Thermodynamically, K_b can be expressed in terms of the ratio of solute activity coefficients in the aqueous phase, γ^w, and in association with bacteria, γ^b.

$$K_b \propto \frac{\gamma^w}{\gamma^b} \qquad (2)$$

The reference state is taken to be the pure liquid (or supercooled liquid) state of the compound at 25°C. If γ^w is assumed to be the distilled water solute activity coefficient, unaffected by the presence of the bacteria, it can be used to relate K_b to other physical properties of the solute.

For hydrophobic solids, γ^w can be related to the mole fraction solubility, X_s [see Prausnitz (5) or Yalkowsky and Valvani (6,7)].

$$\gamma^w = \frac{1}{X_s} \exp \frac{-\Delta S_f}{RT} (T_m - T) \qquad (3)$$

where ΔS_f is the entrophy of fusion and T_m is the melting point. For liquids ($T \geq T_m$), T_m is set at T and the crystal energy component drops out of eq. 3. For the compounds in Table III, $\Delta S_f \sim 13$ eu (7). Therefore, at 25°C the exponential coefficient in eq. 3 reduces to

$$\frac{\Delta S_f}{RT}(T_m - T) = 0.022\,(mp-25) \qquad (4)$$

where now mp is the melting point in degrees Celsius. By combining eq. 2, 3, and 4, K_b can be expressed in terms of solubility:

$$K_b = -\frac{B}{X_s} \exp\,[-0.022\,(mp-25)] \qquad (5)$$

where B incorporates the activity coefficient γ_b as well as the proportionality constant in eq. 2. For hydrophobic solutes, such as those in Table III, γ^w is large (>> 1) and tends to be highly variable from solute to solute, varying roughly as the inverse of the water solubility; this derives largely from solute-solvent dissimilarity. For hydrophobic compounds in association with an organic matrix, however, one would expect smaller values for γ^b (1 to 10) and far less variability between solutes (nearly invariant).

Table III. Biosorption Partition Coefficients and Physical Constants for Condensed Ring Aromatics

Compound	mp°C	K_b	Solubility ppm	Solubility mole fraction	K_{ow}
pyrene	156	2.4×10^4	0.135(11)	1.2×10^{-8}	1.5×10^5(9)
phenanthrene	101	6.3×10^3	1.29(11)	1.3×10^{-7}	3.7×10^4(9)
benz[a]anthracene	160	3.6×10^4(10)	0.014(11)	1.10×10^{-9}	4.1×10^5(9)
benzo[a]pyrene	175	4.5×10^5(10)	0.0038(11)	2.7×10^{-10}	2.2×10^6(9)
benzo[f]quinoline	93	1.5×10^2(10)	76(10)	7.6×10^{-6}	1.6×10^3(9)
7H-dibenzo[c,g]carbazole	157	8.5×10^4(10)	63(10)	4.2×10^{-9}	5.6×10^5(9)
benzo[b]thiophene	31	1.2×10^2(10)	130(10)	1.7×10^{-5}	1.3×10^3(12)
dibenzothiophene	100	1.6×10^3(10)	1.5(2,10)	1.5×10^{-7}	2.4×10^4(2)

For the compounds in Table III, linear least squares fitting of eq. 5, assuming B to be constant, gave

$$K_b = \frac{0.0031}{X_{sol}} \exp\,[-0.022\,(mp-25)] \qquad (R^2 = 0.928) \qquad (6)$$

In this fit, the benzanthracene is the "errant" compound; K_b calculated (eq. 6) deviates from K_b measured by a factor of 4. If this compound is omitted, the quality of fit improves significantly ($R^2 = 0.991$). The conventional log-log regression of K_b and X_s, including the crystal energy term, gave

$$\log K_b = -0.97 \log X_s - \frac{0.022}{2.303}(mp-25) - 2.33 \qquad (R^2 = .964) \qquad (7)$$

The excellence of fit, together with the near unity coefficient for ($-\log X_s$) suggests that the assumed invariance of γ^b was justified.

Another commonly measured physical property that should extrapolate well to K_b is the octanol/water partition coefficient, K_{ow}. Just as was done for K_b (eq. 2), K_{ow} can be expressed in terms of activity coefficients.

$$K_{ow} \propto \frac{\gamma^w}{\gamma^o} \qquad (8)$$

where γ^o is the solute activity coefficient in octanol, saturated with water. If γ^w is again taken to be the distilled water solute coefficient, equations 8 and 2 can be combined to give

$$K_b \propto \frac{\gamma^o}{\gamma_b} K_{ow} \quad (9)$$

As was done previously, one can again rationalize the invariance of γ^o, relative to γ^w, and therefore prescribe that K_b and K_{ow} should be directly proportional. Least-squares fitting the data in Table III to the linear eq. 9 gives

$$K_b = 0.20\ K_{ow} \qquad (R^2 = 0.986) \quad (10)$$

The log-log fit gives

$$\text{Log } K_b = 1.08 \text{ Log } K_{ow} - 1.30 \qquad (R^2 = 0.988) \quad (11)$$

Both the linear and logarithmic K_{ow} equations provide excellent fits of the K_b experimental data. The above logarithmic K_{ow} equation is similar to that generated by Baughman and Paris (1) for a variety of organic pollutants. Again their equation exhibited a slope near unity. It is expected that these equations, both K_{ow} and X_{sol}, will extend to other families of hydrophobic compounds with little or no coefficient adjustment.

In a parallel study, investigating the sorption of hydrophobic pollutants to sediments/-soils (2,8), it was found that sorption partition coefficients, K_p, were highly correlated with organic carbon content of the soil or sediment, and that partition coefficients, normalized to organic carbon, K_{oc} (K_p/fraction organic carbon), were highly sediment/soil independent. Furthermore, excellent estimates of K_{oc} were derived from K_{ow} and X_{sol} (9). A comparison of the resultant equations for K_{oc} and those derived herein for K_b indicates that $K_b \approx 0.5\ K_{oc}$. If K_bs are referenced to organic carbon, instead of total bacterial mass, they would approximate in numerical value K_{oc}s. In other words, for a hydrophobic pollutant, the sorption partition coefficient to bacterial populations derived from natural sediments/soils approximates partition coefficients to sediments/soils, when each is referenced to organic carbon.

SUMMARY

In addition, it is shown that excellent estimates of biosorption partition coefficients for hydrophobic organics can be derived from either an octanol/water partition coefficient, as demonstrated by Baughman and Paris (1), or a combination of water solubility and melting point for the specified organic compound. From the environmental perspective, it is noteworthy that the biosorption coefficients (K_bs) measured for a variety of hydrophobic organic pollutants appear to be similar for the mixed microbial population generated from the sediment/soil systems and independent of their sources.

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(Received in The Netherlands 24 November 1980)